

U.S. ENVIRONMENTAL PROTECTION AGENCY  
OFFICE OF PESTICIDES/HED/SACB/TOX  
ONELINERS

TOXCHEM NO.: 272E

CURRENT DATE: 4/30/92

CITATION	MATERIAL	EPA ACCESSION/ NRID NUMBER	RESULTS	TOX. CAT.	CORE GRADE/ DOCUMENT #
CARCINOGENICITY PEER REVIEW	Cyproconazole		PEER REVIEW DOCUMENT DATE: 4/29/92 CLASSIFICATION: C g		009770

1813



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

009770

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

SUBJECT: Second Carcinogenicity Peer Review of Cyproconazole

FROM:

Linda L. Taylor, Ph.D. and *Linda L. Taylor 4/2/92*  
K. Clark Swentzel  
Review Section II, Toxicology Branch II  
Health Effects Division

and  
Esther Rinde, Ph.D. *E. Rinde 4/2/92*  
Manager, Carcinogenicity Peer Review Committee  
Science Analysis and Coordination Branch  
Health Effects Division (H7509C)

TO:

Carl Grable  
Product Manager #21  
Registration Division (7505C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on January 15, 1992 to re-consider the evidence on Cyproconazole with particular reference to cytotoxicity in the mouse liver and quantification of risk. This was the second CPRC meeting on Cyproconazole. The Registrant submitted a reevaluation of the mouse liver tumor data and raised several issues that they requested be considered regarding Cyproconazole's carcinogenic potential. Additionally, further arguments regarding the adequacy of the dose levels in the rat study were submitted for consideration. The CPRC was not persuaded by the issues/arguments submitted by the Registrant and reiterated its conclusion from the first CPRC meeting on Cyproconazole; i.e., Cyproconazole should be classified as Group C - Possible Human Carcinogen - and that a low dose extrapolation model (Q\*) be used for quantification of potential human cancer risk.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

William L. Burnam

Reto Engler

Karl Baetcke

Marcia Van Gemert

*W. L. Burnam*  
*Reto Engler*  
*Karl Baetcke*  
*Marcia Van Gemert*

Robert Beliles

Robert P. Beliles

009770

Lucas Brennecke

Lucas H. Brennecke

Marion Copley

Marion Copley

Kerry Dearfield

Kerry Dearfield

Esther Rinde

Esther Rinde

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Bernice Fisher

Bernice Fisher

Clark Swentzel

Clark SwentzelLinda L. Taylor<sup>1</sup>Linda L. Taylor

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope Fenner-Crisp

Penelope Fenner-Crisp

Julie Du

Julie Du

George Ghali

G. Ghali

Richard Hill

Richard Hill

Hugh Pettigrew

Hugh Pettigrew

Jean Parker

Jean Parker

William Sette

William Sette

Yin-Tak Woo

Yin-Tak Woo

John Quest

MVG

4. Other Attendees: (Observers)

Eve Andersen (Clament)

3

<sup>1</sup>Also member of Committee for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

## B. Material Reviewed:

The material available for review consisted of previous Peer Review Documents, an Outside Contractor's Evaluation of Carcinogenicity data, and other data summaries prepared by Linda Taylor/Clark Swentzel, and statistical analysis by Bernice Fisher. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Sandoz Corp.

## C. Background Information:

Cyproconazole [ $\alpha$ -(4-chlorophenyl)- $\alpha$ -(1-cyclopropyl-ethyl)-1H-1,2,4-triazole-1-ethanol] is a turf fungicide manufactured by Sandoz Crop Protection Corporation proposed for use on golf courses and sod farms.

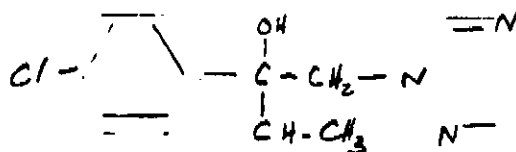
The CPRC first met on June 20, 1990 and classified Cyproconazole as Group C with a  $Q_1^*$ . This decision was based on the increased incidence of liver adenomas and carcinomas in both sexes of treated mice. The first CPRC concluded that the high-dose level tested was inadequate for the carcinogenicity bioassay in rats, as indicated by the lack of any biologically significant body weight decrease, the absence of any histopathological changes accompanying the increase in relative liver weight, and finally the lack of any increase in the liver enzyme activity in females and the inconsistency of such change in the high-dose males. Therefore, the first CPRC recommended that (1) the carcinogenic phase of the rat study be repeated, (2) a range-finding study (90 days) be performed, and (3) the Agency be consulted on dose selection prior to the commencement of the carcinogenic study.

The Registrant responded to the report of the first CPRC and contended that 1) the rat study did use adequate doses, 2) the mouse study should not be used to derive a  $Q_1^*$ , since the tumor incidence was linked to overwhelming cytotoxicity (lytic necrosis), and 3) the substance is not mutagenic.

The Caswell Number of Cyproconazole is 272E

The Chemical Abstracts Registry Number (CAS No.) is .

The structure of Cyproconazole is



009770

D. Evaluation of Carcinogenicity Evidence:

1. Mouse Carcinogenicity Study

Reference: Warren, S., Morand de Jouffrey, S., Muller, F., and Karapaly, J.C. May 31, 1989. The Potential Oncogenicity of SAN 619F by Prolonged Dietary Administration to Mice. Study No. 388-M/398-M, Sandoz AG, Agro Division, Basle, Switzerland. MRID No. 411472-01.

a. Experimental Design

Cyproconazole (technical, 95.1% pure) was fed to Charles River CD-1 mice (50/sex/dose group) at concentrations of 5, 15, 100, and 200 ppm. Females were treated for 88 weeks and males for 81 weeks. Ten animals/sex were assigned to control and high-dose groups for 13-week interim sacrifice. There were two control groups of 50 animals/sex for the entire study.

b. Discussion of Tumor Data

Tables 1 and 2 show the incidences of liver tumors in male and female mice. There were increased incidences of adenomas, of carcinomas, and of adenomas and carcinomas combined. The incidence of liver tumors is not an issue, only whether the tumors would have occurred if Cyproconazole had not caused the severe liver toxicity noted in the study (see below).

c. Non-neoplastic Lesions

The Registrant contends that the liver tumors were only induced in the presence of severe liver toxicity (hepatic lytic necrosis), making these data unsuitable for the development of a Q<sub>1</sub>\*. The second CPRC evaluated the review submitted on the incidence of lytic necrosis (from the Registrant's Outside Contractor's re-review of the liver slides) and determined that the tumor data could not be discounted, since the majority of the animals displaying lytic necrosis, which the Registrant equates to severe liver toxicity, did not display liver tumors and in many cases those displaying liver tumors did not display lytic necrosis.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The first CPRC concluded that the high dose tested was adequate for the assessment of carcinogenic potential.

TABLE 1

Cyproconazole, CD-1 Mouse- Male Hepatocellular Tumor  
Rates<sup>+</sup> and Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>				
<u>Tumor</u>	0 <sup>a</sup>	5	15	100	200
<u>Liver</u>					
<u>Adenomas</u>	6/92	4 <sup>b</sup> /49	5/48	12/47	12/48
(%)	(7)	(8)	(10)	(26)	(25)
p=	0.009**	0.368	0.375	0.013*	0.055
-----					
<u>Liver</u>					
<u>Carcinomas</u>	0/74	0/38	3/46	3 <sup>c</sup> /43	1/41
(%)	(0)	(0)	(7)	(7)	(2)
p=	0.096	1.000	0.031*	0.008**	0.004**
-----					
<u>Both</u>	6/92	4/49	8/48	15/47	13/48
(%)	(7)	(8)	(17)	(32)	(27)
p=	0.003**	0.383	0.086	0.001**	0.022*

<sup>+</sup> Number of tumor bearing animals/ Number of animals examined,  
excluding those that died before observation of the first tumor.

( ) percent

a composed of 2 controls, 50 animals in each one.

b first liver adenoma observed at week 56, dose 5 ppm.

c first liver carcinoma observed at week 68, dose 100 ppm.

Note: Significance of trend denoted at Control.  
Significance of pair-wise comparison with  
control denoted at Dose level.

If \* then  $p < .05$  and if \*\* then  $p < .01$ .

TABLE 2

Cyproconazole- CD-1 Mouse, Female Hepatocellular Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results (p values)

<u>Tumor</u>	<u>Dose (ppm)</u>				
	0 <sup>a</sup>	5	15	100	200
<u>Liver</u> <u>Adenomas</u> (%)	0/61 (0)	0/34 (0)	0/28 (0)	2/41 (5)	6 <sup>h</sup> /39 (15)
p=	0.000**	1.000	1.000	0.067	0.001**
-----					
<u>Liver</u> <u>Carcinomas</u> (%)	0/69 (0)	0/41 (0)	0/31 (0)	0/43 (0)	7 <sup>c</sup> /40 (18)
p=	0.000**	1.000	1.000	1.000	0.000**
-----					
<u>Both</u> (%)	0/69 (0)	0/41 (0)	0/31 (0)	2/43 (5)	13/40 (33)
p=	0.000**	1.000	1.000	0.067	0.000**

<sup>+</sup> Number of tumor bearing animals/ Number of animals examined, excluding those that died before observation of the first tumor.

( ) percent

a composed of 2 controls, 50 animals in each one.

b first liver adenoma observed at week 80, dose 200 ppm.

c first liver carcinoma observed at week 76, dose 200 ppm.

Note: Significance of trend denoted at Control.  
Significance of pair-wise comparison with control denoted at Dose level.

If \* then  $p < .05$  and if \*\* then  $p < .01$ .

## 2. Rat Carcinogenicity Study

Reference: Warren, S.F.P., Carpy, S., and Muller, F. April 22, 1988. SAN 619F Chronic Toxicity/Oncogenicity Feeding Study in Rats. Study No. 357-R, Sandoz Ltd., Agro Development, Basle, Switzerland. MRID No. 411647-01.

### a. Experimental Design

Cyproconazole (technical, 94.6 to 96.6% purity) was fed to 70 KFM-Wistar rats per sex per group at 20, 50, and 350 ppm. These levels correspond to intakes of 1.0, 2.2, and 15.6 mg/kg/day for males and 1.2, 2.7, and 21.8 mg/kg/day for females. Ten rats/sex/dose were sacrificed at 12 and 18 months.

### b. Discussion of Tumor Data

The treatment did not alter the spontaneous tumor profile in this strain of rats.

### c. Non-neoplastic Lesions

There was an increased incidence of fatty change in the liver of the high-dose males.

### d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The decrease in body-weight gain (study termination) at 350 ppm (HDT) was 12.5% in the females and 7.4% in the males, which were not considered biologically significant by the first CPMC. Additionally, although the liver weights were increased and there was vacuolization, there were no life-threatening histopathological lesions or consistent enzyme effects. The first CPMC did not consider the liver to be adversely affected (not accompanied by any significant histopathological lesion) and, since the body-weight gain at 90-days was comparable to the control value, concluded that the high-dose level tested was inadequate for the carcinogenicity bioassay. Therefore, a repeat of the carcinogenicity phase of the rat study was recommended. Following the first CPMC report, the Registrant noted that the terminal body weights may have been affected by the increased liver weights and argued that the decreased body weight gains/microscopic lesions in the liver in the subchronic studies supported their choice of dose levels. The second CPMC found that even when the liver weights were excluded, the body weight changes were still minimal (13.3% in females and 7.75% in males). Additionally, following a review of the data from a subchronic study (4 weeks), in which doses of up to 1000 ppm (10, 30, 100, 1000 ppm) elicited no adverse effects, and a 90-day study, in which the liver histopathological changes at 320 ppm (HDT) were shown to be reversible, the second CPMC concluded that no convincing arguments had been presented by the Registrant to warrant a change in their previous determination that the doses used in the rat study were not adequate for assessing the carcinogenic potential of Cyproconazole.



## E. Additional Toxicology Data on Cyproconazole:

### 1. Metabolism

Metabolism data were submitted after the first CPRC meeting and are classified Core Supplementary, but these are upgradeable with the submission of information verifying the stability of Cyproconazole in DMSO after 14 days.

### 2. Mutagenicity

Cyproconazole has been tested in several mutagenicity studies considered acceptable by the Agency. Since the results of two chromosomal aberration assays indicated that Cyproconazole is clastogenic, the first CPRC requested an additional mutagenicity study (a rodent dominant lethal study) to address an identified heritable risk concern. For the potential to induce chromosome aberrations in CHO cells, Cyproconazole was positive under nonactivated and activated conditions, which supports that Cyproconazole is clastogenic in this test system. Cyproconazole was negative in other assays (Salmonella, mouse micronucleus, and SHE/cell transformation). A dominant lethal assay in rats was submitted, and it was concluded that the assay was negative. Based on this evidence, the concern for a possible heritable effect will not be pursued at this time unless additional evidence to the contrary becomes available. However, the evidence still suggests Cyproconazole has clastogenic activity, and this would add some support to the weight of evidence for carcinogenicity.

### 3. Developmental Toxicity

Developmental toxicity studies in rats and rabbits revealed degeneration of testicular germinal epithelium in mice and dogs; fewer implantation sites, decreased litter sizes and decreased live births in a rat two-generation reproduction study; and decreased number of fetuses and live fetuses/dam, and increased fetal resorptions. There were also indications that the unborn and very young might be sensitive subpopulations with regard to the developmental effects of Cyproconazole. These and other issues regarding developmental effects will be discussed at the Developmental Peer Review Meeting.

### 4. Structure-Activity Correlations

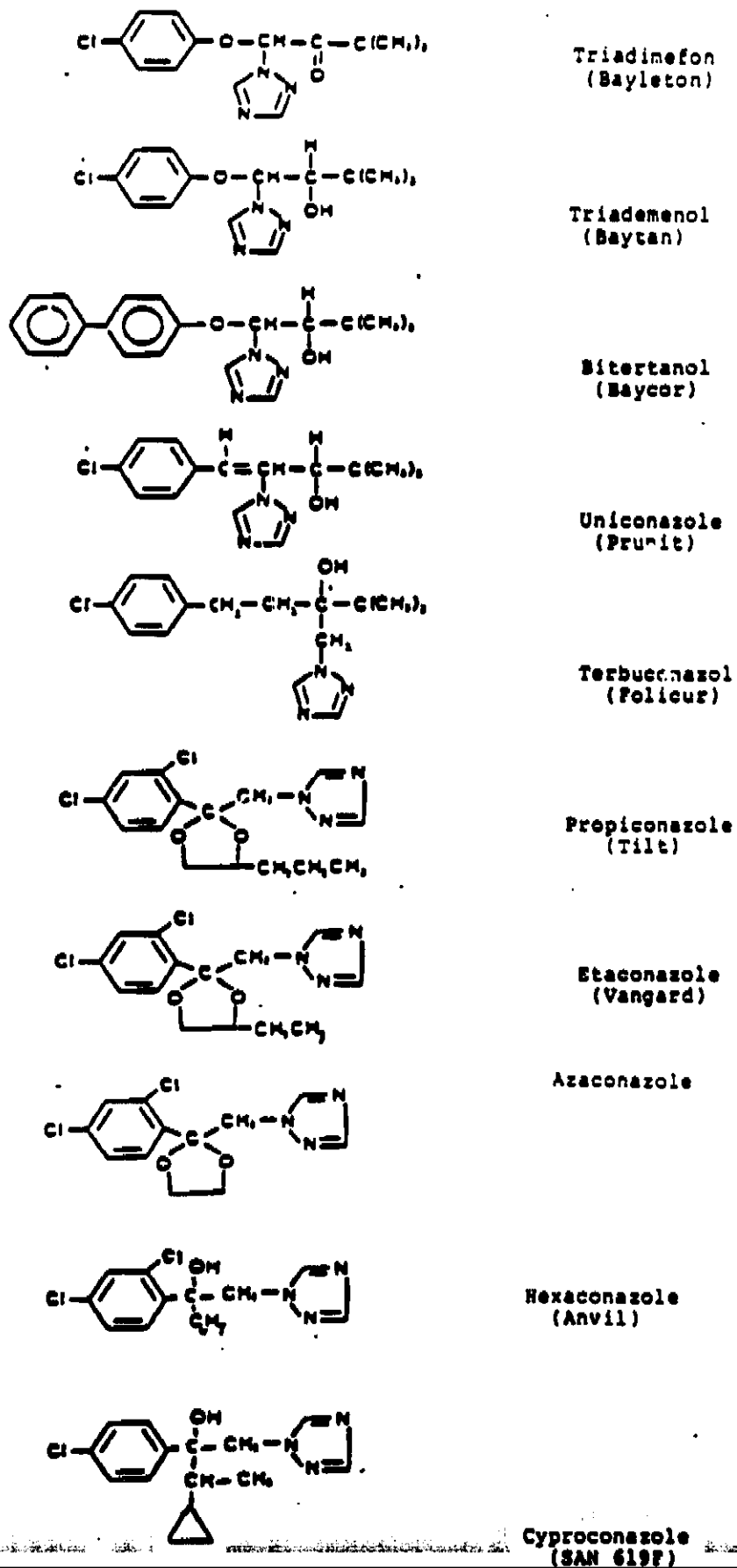
Cyproconazole is structurally similar to other triazole pesticides. These compound structures are in Figure 1 and were discussed in the first PR Document.

Exposure of mice to Triadimefon, Triadimenol, Propiconazole, Etaconazole, and Uniconazole has been associated with an increase in the incidence of liver tumors (dose levels between 1000 and 5000 ppm). Hexaconazole was associated with an increased incidence of benign Leydig cell tumors in the testes of rats, a tumor type not previously reported in response to these structurally-related compounds.

Bitertanol, Azaconazole, Terbuconazole, and Hexaconazole have been reported to be negative for carcinogenicity in mice (dose levels between 180 and 500 ppm). Cyproconazole produced liver tumors in mice at 100 and 200 ppm.

FIGURE I

009770



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Cyproconazole in a weight-of-the-evidence determination of carcinogenic potential.

1. Administration of cyproconazole was associated with an increased incidence of hepatocellular tumors in male and female CD-1 mice. At a dietary dose level of 200 ppm, incidences of carcinomas and of combined adenomas and carcinomas were significantly increased in both sexes. Also at this dose, females had a significantly increased incidence of adenomas. Males had significantly increased incidences of adenomas, carcinomas and of both tumor types combined at the 100 ppm dose, as well as of carcinomas at the 15 ppm dose.
2. In both female and male mice, there were statistically significant positive trends for adenomas and for combined tumors. Female mice showed a statistically significant positive trend for carcinomas also.
3. The increased incidences of hepatocellular tumors noted at 100 and 200 ppm in male CD-1 mice and at 200 ppm in female CD-1 mice generally exceeded the available historical control incidences for these same tumor types in mice of this strain. However, the available historical control data were generated at other laboratories under different conditions and at different time periods. Some of these studies were of longer duration than the cyproconazole study. In these circumstances, the historical control data are considered ancillary information and only minimally confirmatory in nature.

As requested by the Registrant, the CPRC has re-considered the evidence regarding liver toxicity and concluded that the tumor data cannot be discounted, since the majority of the animals displaying lytic necrosis, which the Registrant equates to severe liver toxicity, did not display liver tumors.

4. There was no compound-related increase in tumors observed in male or female rats up to dietary concentrations of 350 ppm. The dose levels fed in the long-term rat study, however, were not adequate to determine the carcinogenic potential of the test material. The Committee recommended that a new rat carcinogenicity study should be submitted.

The information/arguments submitted by the Registrant regarding the adequacy of the dose levels did not alter the Committee's assessment of the study. Review of data from a 4-week study, in which doses up to 1000 ppm elicited no adverse effects, and a 90-day study, in which the liver histopathological changes were shown to be reversible, supported the Committee's previous determination. Additionally, even when the liver weights were excluded from the analysis of body-weight changes, body-weight gains were only slightly reduced and were not considered biologically significant.

5. Although several genetic toxicity tests were negative, an assay on induction of chromosome aberrations in CHO cells was positive under nonactivated and activated conditions. These results indicate that Cyproconazole can be clastogenic without metabolic activation.

This study was repeated and the results support the findings in the first study.

6. The Committee recommended that a rodent dominant lethal study be performed (K. Dearfield, memorandum dated June 20, 1990). This recommendation was based on the fact that administration of Cyproconazole was associated with clastogenic activity, degeneration of testicular germinal epithelium in mice and dogs, fewer implantation sites, decreased live births in a rat two-generation reproduction study, and decreased number of fetuses and live fetuses/dam, and increased fetal resorptions in developmental toxicity studies in rats and rabbits. These results suggest a potential for clastogenic effects and interaction in the germinal tissues.

A dominant lethal study in rats has been performed as requested. The study was adequately performed and the results are negative. Based on this evidence, the concern for a possible mutagenic, heritable effect will not be pursued at this time unless additional evidence to the contrary becomes available.

7. Cyproconazole is structurally related to several triazole pesticide compounds, most of which have been associated with the formation of liver cell carcinomas and/or adenomas in either or both sexes in mice. It should be noted also that Cyproconazole induced this effect at relatively very low doses compared to other triazole pesticides. Also, most of these triazoles were associated with developmental toxicity, particularly of the skeleton.

G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Second Peer Review Committee agreed that the classification for Cyproconazole should remain classified as Group C - possible human carcinogen and agreed with the first recommendation that, for the purpose of risk characterization, a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (Q<sub>1</sub>\*).

This decision was based on the fact that a statistically significant increase in the incidence of carcinomas and combined carcinomas/adenomas was found at the HDT in male and female mice. These tumors are of a malignant nature, are considered rare in females, and were induced in both sexes at a relatively low dose compared to other triazole pesticides. The mouse study was conducted using adequate doses for the determination of carcinogenic activity.

Cyproconazole is structurally related to other carcinogenic triazole pesticides, and there is some evidence that it has clastogenic activity.

The second CPRC agreed with the previous recommendation for retesting in both sexes of the rat since the doses used in the previous rat study were not adequate to determine its carcinogenic potential. A range-finding (90-days) study should be performed, and the Agency should be consulted on dose selection prior to the commencement of this rat carcinogenicity study. This was the same recommendation made at the time of the first Peer Review Committee Meeting (Memo, 6/20/90).

**END**